On-treatment function testing of platelets and long-term outcome of patients with peripheral arterial disease undergoing transluminal angioplasty

van der Loo, B ; Braun, J ; Koppensteiner, R

Abstract: OBJECTIVE: To assess the clinical importance of on-treatment function testing of platelets in patients on aspirin after catheter-based vascular interventions. MATERIALS AND METHODS: In 109 patients with symptomatic peripheral arterial disease (PAD) of the lower limbs, platelet function testing (adenosine diphosphate-, collagen- and epinephrine-induced aggregation using light transmission aggregometry) was performed before and at multiple time points up to 1 year after a percutaneous angioplasty. Using univariate mixture models and Box-Cox transformation to ensure normally distributed individual variances, we investigated if an intraindividual variability exists and if it has a consequence for clinical outcome. RESULTS: Response to aspirin as measured by platelet aggregometry varies considerably over time in most patients. However, the intraindividual variance over time was not significantly correlated either with restenosis/reocclusion after 1 year or with adverse long-term outcome (occurrence of death for cardiovascular cause, stroke or myocardial infarction in up to 8 years follow-up). CONCLUSIONS: Response to aspirin does not seem to have a role in determining long-term outcome in patients with symptomatic PAD. The fact that testing of platelet function at only one time point has reduced significance may have implications for all clinical settings in which aspirin is used for the prevention of thrombo-embolic events.

DOI: https://doi.org/10.1016/j.ejvs.2011.08.014

On-treatment function testing of platelets and long-term outcome of patients with peripheral arterial disease undergoing transluminal angioplasty

Bernd van der Loo*, Julia Braun†, and Renate Koppensteiner*

*Clinic of Angiology, Department of Medicine, University Hospital Zurich, Switzerland, and

† Biostatistics Unit, Institute for Social and Preventive Medicine, University of Zurich, Switzerland

Short title: Response to aspirin and peripheral arterial disease

Category: Original article, Peripheral arterial disease

Address for correspondence:
Renate Koppensteiner, M.D.
Professor of Medicine
Head, Division of Angiology
Department of Internal Medicine II
Medical University of Vienna
Währinger Gürtel 18 – 20
A – 1090 Vienna, Austria
Phone: 0043 1 40 400 / 4670
Fax: 0043 1 40 400 / 4665
E-mail: **renate.koppensteiner@meduniwien.ac.at**

Abstract

Objective: To assess the clinical importance of on-treatment function testing of platelets in patients on aspirin after catheter-based vascular interventions.

Materials and Methods: In 109 patients with symptomatic peripheral arterial disease (PAD) of the lower limbs, platelet function testing (ADP-, collagen- and epinephrine-induced aggregation using light transmission aggregometry) was performed before and at multiple time points up to one year after a percutaneous angioplasty. Using univariate mixture models and Box-Cox transformation to ensure normally distributed individual variances we investigated if an intraindividual variability exists and if it has a consequence for clinical outcome.

Results: Response to aspirin as measured by platelet aggregometry varies considerably over time in most patients. However, the intraindividual variance over time was neither significantly correlated with restenosis/reocclusion after one year nor with adverse long-term outcome (occurrence of death for cardiovascular cause, stroke, or myocardial infarction in up to eight years follow-up).

Conclusions: Response to aspirin does not seem to have a role in determining long-term outcome in patients with symptomatic PAD. The fact that testing of platelet function at only one time point has reduced significance may have implications for all clinical settings in which aspirin is used for the prevention of thromboembolic events.

Keywords: aspirin, platelet function, peripheral arterial disease, restenosis, angioplasty

Introduction

Aspirin (acetylsalicylic acid; ASA) is one of the most important drugs used for the prevention of thromboembolic vascular events. A highly variable interindividual platelet response to the treatment with aspirin has been reported.[1] "Aspirin resistance" is defined differently by different groups. Clinically, "aspirin resistance" or hyporesponsiveness occurs in patients who, although being on therapeutic doses of aspirin, develop thromboembolic cardiovascular events.[2] Others, however, have supported not including "thromboembolic events while on aspirin" as a clinical definition for aspirin resistance as only about one fourth of all vascular complications can be prevented by any one pharmacological therapy alone.[3]

Biochemical assessment of platelet function may detect hypo- or non-response of platelets to aspirin therapy. However, to date, it is not clear which of several available laboratory methods has the best reliability and, in particular, the highest predictability for clinical events.[4] A recent meta-analysis of 20 studies using different aggregation tests to assess individual platelet response to aspirin demonstrated an elevated cardiovascular risk associated with aspirin resistance.[5] Therefore, although the clinical importance of non-responsiveness is not yet fully understood, there is evidence for its clinical relevance. Furthermore, in patients with cerebrovascular ischemic events being on aspirin for secondary stroke prophylaxis, non-responsiveness was significantly associated with recurrent ischemia.[6]

Peripheral arterial disease (PAD), in particular of the lower limbs, is highly prevalent especially in the elderly population.[7] Its utmost importance as a marker of atherosclerotic vascular disease in general has been confirmed.[8]

Percutaneous transluminal angioplasty (PTA) to revascularize peripheral arteries is an established treatment in symptomatic patients. Aspirin is given routinely to patients being treated for peripheral arterial disease and has been shown to reduce the risk of reocclusion after vascular procedures.[9] Therefore, response to aspirin in this setting is of great importance.

A previous study analyzing the time course of agonist-induced platelet aggregation in patients who underwent PTA of the lower limbs provided inconclusive results and was only limited to a 12 months' follow-up.[10]

Here, we wanted to investigate if, apart from the known variable interindividual platelet response to aspirin, an intraindividual variability also exists, and, if so, whether such an intraindividual variance has a consequence for clinical outcome. To this end, individual variances were correlated first with the occurrence of restenosis/reocclusion in patients after PTA of the lower limbs within one year. Secondly, we tested the hypothesis that defined clinical endpoints (e.g. cardiovascular events such as myocardial infarction, stroke, peripheral re-intervention) during long-term follow up of up to almost eight years are more often reached in patients with a high variance.

Materials and methods

Patients

The current study was done as a substudy of a previously published trial.[11] Over a three-year-period (January 1999 until December 2001) 109 of 250 patients screened patients with symptomatic (Fontaine clinical stage II-IV) PAD of the lower limbs, scheduled to undergo PTA at the Clinic of Angiology, University Hospital Zurich, were enrolled. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki. All patients gave their written informed consent. Age, sex, cardiovascular risk factors, history and current medication were recorded at baseline. Characteristics of the lesions were recorded and classified according to the TransAtlantic interSocietal Consensus (TASC) classification.[12] Basic treatment was 100mg aspirin daily which was initiated the day before the procedure if the patient was not already on aspirin (which was the case in all but two patients). Adjunctive drug therapy during the intervention (unfractionated or low-molecular-weight heparin) was at the operator's discretion. Patients were excluded if they were taking anticoagulants at the time of admission, had an intolerance to aspirin, active bleeding or hemorrhagic diathesis. Patients receiving non-steroidal anti-inflammatory agents (NSAID) were also excluded. Furthermore, patients with renal failure, uncontrolled arterial hypertension, recent surgery of the central nervous system, nonatherosclerotic vascular disease, lesions in femoropopliteal bypass grafts, stent implantation and stroke, were excluded, too. At time of admission, routine laboratory tests (complete blood cell count, hematocrit, serum creatinine, INR, cholesterol, triglycerides) were performed using standard methods.

Mean follow-up was 80 months (range 52-94 months). Owing to the parallel group design of the original trial,[11] patients had initially been randomized to one of two treatment arms (no additional treatment versus dalteparin, 5000 IU subcutaneously once daily on day 1 and 2 after the intervention followed by 2500 IU once daily for 90 days in addition to aspirin 100

mg daily). Unpaired t-test revealed that, after three months, neither for ADP ($p = 0.4942$, ns), nor for epinephrine ($p = 0.9594$, ns) or collagen ($p = 0.5541$, ns) any significance of an influence of dalteparin on parameters of platelet aggregation could be observed. Therefore, patients that had received dalteparin within the first three months after angioplasty were also included into the current study.

Follow-up and end points

ADP-, collagen-, and epinephrin- induced platelet aggregation was assessed before, and at 1, 3, 6, and 12 months after the percutaneous procedure. Patients were followed-up for 80 months (range 52-94 months). The day after the procedure as well as at 1, 3, 6, and 12 months after PTA, all patients were examined clinically, and pulse volume recordings, determination of ankle brachial index (ABI), as well as colour-coded duplex sonography were performed. Furthermore, drug use, in particular NSAID and/or the addition of a thienopyridine, was monitored at the time of out-patient testing. Testing of platelet function was always performed under fasting conditions at the same time of the day (early in the morning). Long-term follow-up was based on patient chart review in the majority of the cases. If this was not available, a telephone interview was performed.

Outcome measures were the primary endpoint of restenosis/reocclusion in the dilated segment within one year and the secondary (combined) endpoint consisting of one or more of the following clinical endpoints during long-term follow-up: occurrence of a non-fatal cardiovascular event (myocardial infarction, stroke); death for cardiovascular cause; need for an invasive (either surgically or catheter-based) vascular intervention in any peripheral artery; worsening of clinical symptoms as documented by a drop in ABI of > 0.1.

Testing of platelet function

ADP-, collagen-, and epinephrin- induced platelet aggregation were assessed by light transmittance aggregometry (LTA). In the fasting and resting patient, blood was taken from an antecubital vein via i. v. cannula (1.2 mm) directly into silicon-coated vacutainer tubes containing 0.5 ml buffered sodium citrate (3.8 %). Samples were processed immediately, and platelet aggregation was measured after centrifugation (100 G, 10 min, room temperature) of citrated blood (3.8%; 1:10) to get platelet-rich plasma (PRP). In addition, the remaining sample was further centrifuged at a higher rate (3000 G, 15 min, RT) to obtain platelet pure plasma (PPP). To determine platelet aggregation to the different agonists, a standardized plasma sample with a total platelet count of 250 G/ l was used by adjusting PRP with PPP. Platelets were stimulated with epinephrine (final concentration 0.1 mmol/l), collagen (final concentration 5 mg/l) and ADP (final concentration 0.002 mol/l), and aggregation was measured using an APACT 4 aggregometer (Ahrensburg, Germany). Aggregation was expressed as the maximum percentage change in light transmittance from baseline.
The coefficient of variation (CV) for LTA in our laboratory was 8.9% (n=20).

Statistical analysis

For data management and analysis the statistical software package R, version 2.6.1 (R Development Core Team, Vienna, Austria) was used. Continuous variables are reported as means ± SD, categoric variables as counts and percentages. Pearson's correlation was used to check if two continuous variables were strongly correlated, so that they could be treated as exchangeable. The Mann Whitney *U* test was used for comparisons of continuous variables, the chi-squared test for comparisons of categorical variables. A p value < 0.05 was considered to be statistically significant.

Univariate mixture models: For each timepoint and each of the three measures (ADP, collagen, epinephrine) we first fit three different univariate models: one using the ordinary

normal distribution, one using a mixture of two normal distributions with equal variances and one using a mixture of two completely distinct normal distributions. The different models were calculated using an expectation-maximization (EM) algorithm. They were compared graphically and via the Akaike's information criterion (AIC). The model with the smallest AIC for each timepoint has been selected.

Box-Cox-transformation: To compare variances of ADP-, collagen-, or epinephrine-induced platelet aggregation over time between patients with and without restenosis and between patients reaching one of the endpoints and those who did not, a t-test can only be performed if these variances are approximately normally distributed in both groups. As this was not the case, we used the Box-Cox transformation to achieve normality. We decided to use the quartic root (i.e. the squareroot of the standard deviation) of the variances of ADP, collagen, and epinephrine. Furthermore, in order to appropriately incorporate the fact of variable numbers of measurements in different individuals into the analyses, a weighted t-test was used. To avoid the problem of multiple testing, Bonferroni correction was used.

Results

Baseline demographic and clinical characteristics as well as laboratory variables are shown in

Table 1A and 1B.

Patients were grouped according to the occurrence of restenosis/reocclusion within 12 months (**table 1A**) as the primary endpoint and according to the occurrence of any fatal or non-fatal cardiovascular event and/ or the need for any surgically or catheter-based vascular intervention (i.e. secondary endpoint) during complete long-term follow-up (**table 1B**). As expected, patients with restenosis/reocclusion had a lower ankle-brachial index than those without restenosis/reocclusion within the first year (0.64±0.17 vs. 0.74±0.19; p=0.0077) (**table 1A**). Patients reaching the combined endpoint were slightly younger than those who did not (68±11 years vs. 72±10 years; p=0.046) (**table 1B**). Apart from these differences, the groups were similar with respect to their baseline parameters.

Mean values of ADP-, epinephrine- and collagen-induced platelet aggregation, as assessed by LTA, for all patients up to one year after the initial percutaneous procedure are shown in

Table 2.

When comparing these values between patients with any event and those without during long-term follow-up, no significant differences could be detected.

Only 25.6% of the patients were sampled at 12 months, and 52 % of all patients had their agonist-induced platelet aggregation measured at ≥ three timepoints (**table 2**). However, in order to circumvene this problem, and to analyze individual variances in the best possible way under these circumstances, a weighted t-test was performed.

We then wanted to investigate if subgroups exist, i.e. patients whose values for platelet aggregation upon stimulation are at all timepoints higher or lower than the average. To this end, we first plotted all data separately for each individual.

Figure 1.

There was a considerable amount of variation in most of the individuals. As outlined above, we therefore tried to fit mixture distributions in the univariate (i. e. for all timepoints separately) case. For each timepoint we chose for ADP (epinephrine and collagen not shown) the best out of three possible univariate models (normal distribution; mixture of two normal distributions with equal variances; mixture of two distinct normal distributions). A mixture of two normal distributions with distinct variances was chosen for time points 0 and 12. A mixture of two normal distributions with equal variance was chosen for timepoints 3 and 6. One normal distribution was chosen for timepoint 1.

Figure 2.

We found there was no distinct subgroup detectable which would remain the same over the observed time period.

We also calculated correlations of the agonists ADP, epinephrine and collagen for all five timepoints. Although there was a positive correlation remaining relatively constant over time, this correlation is not strong (between r=0.25 and r=0.64). Therefore, we did not treat the agonists as exchangeable.

We then wanted to analyze whether the initially observed high variance of ADP-, epinephrine-, and collagen- induced platelet aggregation within any individual patient might have a significant influence on the occurrence of restenosis/reocclusion (within one year after PTA) and/ or the combined endpoint (during complete long-term follow-up). Results of a weighted t-test are shown in

Table 3.

In all but one constellation there was no evidence for a difference between the groups. Only for the time course of collagen, a p-value below 0.05 indicated a significant difference with respect to the occurrence of restenosis/reocclusion. However, in this context, the multiple testing problem has to be borne in mind, so that the probability of a wrong significant result cannot be controlled via $\alpha = 0.05$ any more. The appropriate level of significance resulting

from the Bonferroni correction is 0.008. Therefore, no evidence for a difference between the groups could be found.

Discussion

In our study with its unique aspects of multiple time points of platelet function testing and a very long clinical follow-up we found that platelet aggregation results using light transmission aggregometry in symptomatic patients with PAD intraindividually vary considerably over time and do not correlate with long-term outcome. To the best of our knowledge, this is the first study to describe this. Others have recently reported a high intra-individual variability of ADP- and collagen- induced platelet aggregation.[13] However, these findings were observed in healthy individuals receiving aspirin for up to eight weeks only whereas we describe this for the first time in a pathophysiologic context. Furthermore, we had multiple time points for platelet function testing during a long period of twelve months and correlated our findings with clinical endpoints during a follow-up of up to eight years.

Because of the missing values especially for the later timepoints, it was difficult to fit complex mixture distributions. However, we could circumvene this problem by using a weighted t-test. We cannot rule out that variable numbers of measurements in different individuals might possibly have affected our data on the correlation between intraindividual variance and long term cardiovascular outcome. However, our main conclusion (i.e. that testing of platelet function at one timepoint only has reduced significance) remains unaffected.

This finding may potentially entail serious implications, as it means that on-treatment function testing of aspirin, done at one timepoint, usually before a peripheral or coronary intervention in certain patients at individually high risk for thrombosis, has a reduced significance, at least when LTA is used. This could also mean that a correlation between response to aspirin and clinical events (as shown in several studies in the past) has to be interpreted with caution.

Low-dose daily aspirin is a widely accepted strategy for secondary prevention in patients with vascular disease. It is still a matter of controversy if the biochemical detection of hypo- or non-responsiveness to aspirin has a clinical meaning, i. e. an association with thrombotic

events. However, in our study we could not show that the individually high variance translates into a higher risk for restenosis/reocclusion after PTA or for the occurrence of a major cardiovascular event.

We further found no obvious hint for the existence of subgroups of patients in which platelet aggregation testing revealed a lack of response to aspirin at all time points. With univariate mixture models fit to find the correct location for each individual within the mixture clearly separated patients could not be identified.

An interesting hypothesis which arises from our work is that a single patient being a responder to aspirin once will not always be a responder.

In this context, the different etiologies for "aspirin resistance" have to be borne in mind. One of the most important reasons for "aspirin resistance" is a lack of compliance.[14, 15] However, virtually all our patients had been on aspirin at the beginning of the study. The variation in platelet response to agonists as depicted in figure 1 would imply a high degree of irregularity in taking aspirin which makes this explanation completely speculative. In a previous study platelet hyporesponsiveness to aspirin was evaluated in 24 patients at day ten and one month after coronary artery bypass graft surgery.[16] The authors found that although most patients could be classified as "aspirin-resistant" shortly after their operation, responsiveness to aspirin was restored in a majority of the patients at one month' follow-up .[16] We used in the present work a completely different study design in a different group of patients. Furthermore, we here describe a high degree of individual variance in the platelet responsiveness to aspirin rather than a restoration of platelet function. However, our data also point to a transient nature of response to aspirin, implying its modifiability.

In this study, we did not aim to identify possible modulating factors nor to tackle the question whether a defective action of aspirin on the arachidonic acid pathway, insufficient bioavailability, inadequate, i. e. too low dosage or a progression of peripheral artery disease

are causally involved.[17, 18] However, none of the afore mentioned causes could really explain the phenomenon described here.

Platelet alterations have previously been described in patients with peripheral arterial disease.[19] Thus, it is conceivable that platelet-plaque interaction and/or platelet-injured endothelial cell interaction may modulate platelet function to a variable degree, depending on the actual state of disease, influenced by inflammatory processes.

Strong evidence linking "aspirin resistance" assayed ex vivo and the future risk of myocardial infarction, stroke or cardiovascular death came from a nested case-control study on a subgroup of patients enrolled in the HOPE study.[20] In the current study, it was not our principal aim to merely correlate response to aspirin with clinical long-term outcome.

Interestingly, in a previous reproducibility study specifically designed to measure platelet aggregation over time, very consistent results were found in a small proportion of individuals exhibiting an unusual hyperreactivity to agonists.[21] However, this study had been performed in healthy volunteers not being on aspirin.

Our results cast doubt on the significance of platelet function testing using LTA in patients while being on aspirin when assessed at one timepoint only. Our data may imply that the modalities of such tests will have to be refined in the future.

Disclosure of conflict of interest:

The Authors declare no competing financial interests.

References

1. Storey RF. Variability of response to antiplatelet therapy. Eur. Heart J. Supplements 2008; 10 (suppl A): A21-A27.
2. Gengo FM, Rainka M, Robson M, Gengo MF, Forrest A, Hourihane M, et al. Prevalence of platelet nonresponsiveness to aspirin in patients treated for secondary stroke prophylaxis and in patients with recurrent ischemic events. J. Clin. Pharmacol. 2008; 48: 335-343.
3. Patrono C, Rocca B. Drug insight: aspirin resistance – fact or fashion? Nat. Clin. Pract. Cardiovasc. Med. 2007; 4: 42-50.
4. Lordkipanidze M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur. Heart J. 2007; 28: 1702-1708.
5. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. Br. Med. J. 2008; 336: 195-198.
6. Pamukcu B. A review of aspirin resistance: definition, possible mechanisms, detection with platelet function tests, and its clinical outcomes. J. Thromb. Thrombolysis 2007; 23: 213-222.
7. Diehm C, Schuster A, Allenberg JR, Darius H, Haberl R, Lange S, et al. High prevalence of peripheral arterial disease and co-morbidity in 6880 primary care patients: cross-sectional study. Atherosclerosis 2004; 172: 95-105.
8. Diehm C, Lange S, Darius H, Pittrow D, von Stritzky B, Tepohl G, et al. for the getABI Study Group. Association of low ankle brachial index with high mortality in primary care. Eur. Heart J. 2006; 27: 1743-1749.

9. Antiplatelet Trialists Collaboration. Collaborative overview of randomized trials of antiplatelet therapy. II. Maintenance of vascular graft or arterial patency by antiplatelet therapy. Br. Med. J. 1994; 308: 159-168.
10. Mueller MR, Salat A, Stangl P, Murabito M, Pulaki S, Boehm D, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. Thromb. Haemost. 1997; 78: 1003-1007.
11. Koppensteiner R, Spring S, Amann-Vesti B, Meier T, Pfammatter T, Rousson V, et al. Low-molecular-weight heparin for prevention of restenosis after femoropopliteal percutaneous transluminal angioplasty: a randomized controlled trial. J. Vasc. Surg. 2006; 44: 1247-1253.
12. Dormandy JA, Rutherford RB. Management of peripheral arterial disease (PAD). TransAtlantic interSociety Consensus (TASC). J. Vasc. Surg. 2000; 31: S1-S296.
13. Santilli F, Rocca B, de Cristofaro R, Lattanzio S, Pietrangelo L, Hybib A, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays. Implications for “aspirin resistance”. J. Am. Coll. Cardiol. 2009; 53: 667-677.
14. Weber AA, Przytulski B, Schanz A, Hohlfeld T, Schrör K. Towards a definition of aspirin resistance: a typological approach. Platelets 2002; 13: 37-40.
15. Schwartz KA, Schwartz DE, Gosheh K, Reeves J, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. Am. J. Cardiol. 2005; 95: 973-975.
16. Golanski J, Chlopicki S, Golanski R, Gresner P, Iwaszkiewicz A, Watala C. Resistance to aspirin in patients after coronary artery bypass grafting is transient. Impact on the monitoring of aspirin antiplatelet therapy. Ther. Drug Monit. 2005; 27: 484-490.
17. Trenk D, Neumann FJ. Aspirin resistance. J. Am. Coll. Cardiol. 2008; 52: 740-742.

18. Gasparyan AY, Watson T, Lip GY. The role of aspirin in cardiovascular prevention: implications of aspirin resistance. J. Am. Coll. Cardiol. 2008; 51: 1829-1843.

19. McBane II RD, Karnicki K, Miller RS, Owen WG. The impact of peripheral arterial disease on circulating platelets. Thromb. Res. 2004; 113: 137-145.

20. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002; 105: 1650-1655.

21. Yee DL, Sun CW, Bergeron AL, Dong JF, Bray PF. Aggregometry detects platelet hyperreactivity in healthy individuals. Blood 2005; 106: 2723-2729.

Table 1: Baseline demographic and clinical characteristics of all patients by endpoints

A

	restenosis/ reocclusion (n= 48)	**no restenosis/ reocclusion** (n=61)	**p**
Age (years)	68±11.3	70±10.2	0.377
Male	25 (52%)	36 (59%)	0.353
Diabetes	16 (33%)	18 (30%)	0.755
Hypercholesterolemia	35 (73%)	41 (67%)	0.565
Arterial Hypertension	37 (77%)	50 (82%)	0.439
Pack years	32.98±29.1	34.33±40.0	0.848
History of coronary heart disease	16 (33%)	26 (43%)	0.258
History of cerebrovascular disease	8 (17%)	6 (10%)	0.322
TASC classification			0.207
A	9 (19%)	12 (20%)	
B	10 (21%)	22 (36%)	
C	21 (44%)	22 (36%)	
D	8 (17%)	5 (8%)	
Ankle-brachial index	0.64±0.17	0.74±0.19	**0.0077**
Platelet count ($x10^3/\mu l$)	256±73	265±75	0.544
Fibrinogen (mg/dl)	396±97	381±79	0.401

B

	with events* (n=66)	**without events*** (n=43)	**p**
Age (years)	68.1±11.4	72.3±9.7	**0.046**
Male	37 (56%)	24 (56%)	0.979
Diabetes	23 (35%)	11 (26%)	0.307
Hypercholesterolemia	50 (76%)	27 (63%)	0.146
Arterial Hypertension	51 (77%)	37 (86%)	0.256
Pack years	36.4±38.8	27.9±28.2	0.234
History of coronary heart disease	24 (36%)	18 (42%)	0.564
History of cerebrovascular disease	11 (1.5%)	3 (7%)	0.139
TASC classification			0.722
A	11 (17%)	10 (23%)	
B	19 (29%)	13 (30%)	
C	26 (39%)	16 (37%)	
D	10 (15%)	4 (9%)	
Ankle-brachial index	0.68±0.19	0.71±0.19	0.514
Platelet count ($x10^3/\mu l$)	270±78	249±66	0.156
Fibrinogen (mg/dl)	392±94	386±80	0.740

*events = clinical endpoints (myocardial infarction, stroke, peripheral re-intervention at any site; death for cardiovascular cause)

Table 2: ADP-, epinephrine- and collagen- induced platelet aggregation according to the secondary endpoint in PAD patients (n=109) on aspirin before and during a follow-up period of twelve months after PTA.
Values are means ± SD, in %.

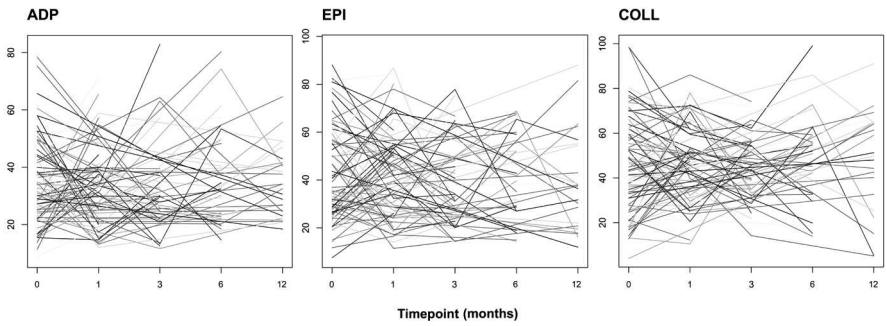
	with events* (n=66)	without events* (n=43)	p
ADP			
Baseline (n=109)	35.2 ±15.1	34.6±15.0	0.839
1 month (n=57)	32.4±12.7	33.5±15.4	0.777
3 months (n=39)	31.7±14.4	31.9±17.1	0.964
6 months (n=28)	38.2±19.6	35.7±9.4	0.716
12 months (n=28)	35.9±11.9	32.6±12.0	0.480
Epinephrine			
Baseline (n=108)	42.1±18.5	43.6±20.3	0.701
1 month (n=57)	41.0±20.6	41.8±16.5	0.879
3 months (n=38)	40.0±18.8	34.7±18.3	0.382
6 months (n=28)	41.2±19.3	41.6±14.9	0.963
12 months (n=24)	43.0±24.1	34.8±13.4	0.342
Collagen			
Baseline (n=108)	45.0±20.4	48.8±21.2	0.357
1 month (n=55)	45.6±18.3	52.8±18.7	0.176
3 months (n=38)	50.3±13.9	42.5±15.0	0.103
6 months (n=28)	50.3±21.9	48.2±16.6	0.802
12 months (n=22)	48.3±24.6	48.0±21.3	0.976

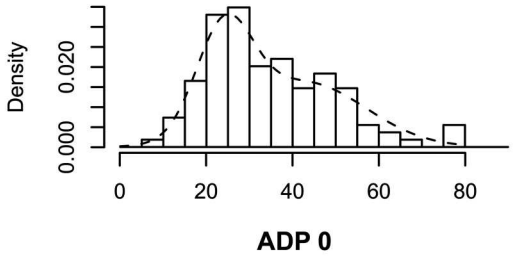
*events = clinical endpoints (myocardial infarction, stroke, peripheral re-intervention at any site; death for cardiovascular cause)

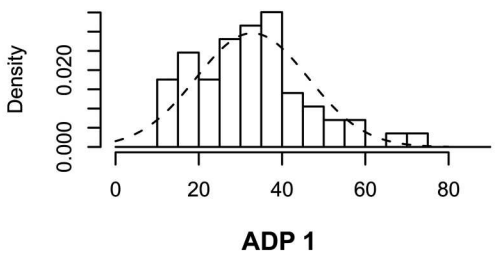
Table 3: Point estimates, confidence intervals and p-values for a weighted t-test for differences of the quartic root of the variances for ADP-, epinephrine- and collagen-induced platelet aggregation. Restenosis indicates restenosis/reocclusion of the dilated segment within one year, event indicates the secondary (=combined) endpoint during long-term follow-up.

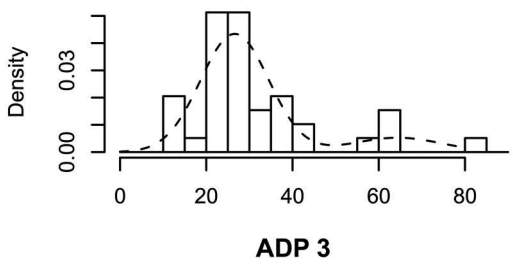
	point estimate	Lower limit	upper limit	p
ADP, restenosis/ reocclusion	-0.087	-0.632	0.458	0.751
ADP, event*	0.011	-0.520	0.542	0.967
COLL, restenosis/ reocclusion	-0.551	-1.035	-0.067	0.026
COLL, event*	-0.116	-0.613	0.380	0.643
EPI, restenosis/ reocclusion	0.129	-0.365	0.622	0.605
EPI, event*	0.411	-0.067	0.889	0.091

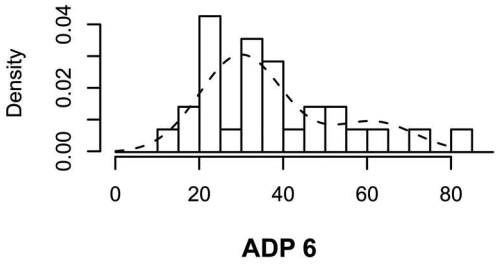
*event = clinical endpoints (myocardial infarction, stroke, peripheral re-intervention at any site; death for cardiovascular cause)

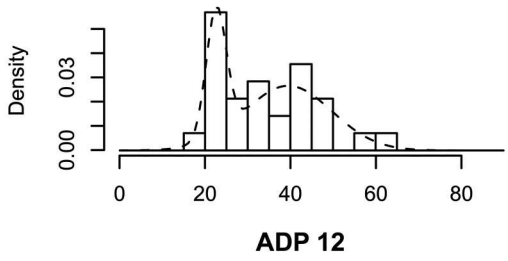

ADP
EPI
COLL
Timepoint (months)


Density
0.000
0.020
0
20
40
60
80
ADP 0


Density
0.000
0.020
0
20
40
60
80
ADP 1


Density
0.00
0.03
0
20
40
60
80
ADP 3


Density
0.00
0.02
0.04
0
20
40
60
80
ADP 6


Density
0.00
0.03
0
20
40
60
80
ADP 12

Legends to figures

Figure 1: Platelet aggregation to ADP, collagen and epinephrine separately for each individual at five different timepoints (n=109). There are variable numbers of measurements per individual.

Figure 2: Distribution of values for ADP-induced platelet aggregation over time.
0 indicates baseline, 1, 3, 6, and 12 indicate months of follow-up. For each timepoint, the best of three possible fitting models (mixture of two completely distinct normal distributions; mixture of two normal distributions with equal variances; model with just one normal distribution) was chosen. For a detailed description see Methods.